Field Evaluation of Rhizobium Inoculant Formulations for Alfalfa

J. D. Knight

Saskatchewan Centre for Soil Research, University of Saskatchewan,
Saskatoon, SK, S7N 5A8

Keywords: alfalfa, forages, granular inoculants, *rhizobium meliloti*

Abstract

In an on going study, granular *Rhizobium* inoculants for alfalfa are being compared to pre-inoculated seed and on-site peat inoculated seed for their effects on alfalfa stand establishment, nodulation, forage yield and biological nitrogen fixation. In addition, depth of inoculant placement is being investigated. Field sites were established in 1999 at three locations in the alfalfa production area of Saskatchewan. Treatment effects varied by site, however in general on-site inoculation resulted in inferior stand establishment and forage yield in the establishment year. Plots where granular inoculants were banded below the alfalfa seed row generally had the best stand establishment and yields in the establishment year. Treatment differences disappeared by the first cut of the first production year. Thus, although granular inoculants may lead to superior establishment they do not appear to have a long term benefit. They study will continue for a third year.

Introduction

Rhizobium inoculants formulated as clay or peat granules are increasing in popularity with producers in western Canada for inoculating pulse crops. Granular formulations are preferred by some producers because they eliminate the on-site mixing of the inoculant with the seed during the busy seeding period. Granular inoculants are soil placed in the same manner as fertilizers and are applied through a traditional fertilizer box. In addition to the ease of handling, a number of studies on pulse crops have shown these granular formulations to be superior to seed applied inoculants in their ability to cause nodulation and subsequent biological nitrogen fixation (Bezdicek et al., 1978; Muldoon et al., 1980; Walley pers. commun.). It is thought that this superior nitrogen fixation ability is associated with the fact that granular formulations can be placed deeper in the soil, offering protection to the bacteria from drying and moisture fluctuations (Smith, 1992). Additionally, because the root must grow toward the inoculant supply, more of the secondary root system becomes infected and ultimately nodulated rather than simply the primary tap root. With seed applied inoculants, during germination the tap root is the first organ` to encounter the seed adhered bacteria and becomes the primary site of nodulation.

Granular *Rhizobium* formulations currently are not commercially available for forage legumes. In theory, the features that make them successful and popular as pulse crop inoculants should apply equally to forage legumes. Because of the small seed size of forage legumes like alfalfa and clover, these crops must be seeded very shallowly in the soil. Shallow seeding increases the risk of exposure of the bacteria to the atmosphere thereby increasing the probability of drying and bacterial death. Granular placement below the seed row should increase bacterial viability.

Furthermore, unlike pulse seed which has a large surface area for bacterial adhesion, the small size of forage legumes limits the numbers of bacteria that can adhere to a single seed (Clayton et al., 1996). Granular formulations enable the rate of delivery of the bacteria to the soil to be varied independently of the seeding rate.

Alfalfa typically is seeded along with a companion crop, like oat or canola, in the establishment year. Although companion crops compete with forage seedlings for resources they also provide some protection for alfalfa seedlings from heat, wind and water and suppress weeds. Furthermore, they provide some production from the field in the year of establishment but usually reduce subsequent yields of the forage (SAF 1998). In the appropriate management system, an alternative for delivering rhizobia to the soil may be to inoculate the seed of the companion crop, thereby using the companion crop as the delivery system for the inoculant. This only would apply to large seeded companion crops (like oat) that are seeded deeper in the soil than the alfalfa. Inoculation of the oat seed rather than the alfalfa seed would place the *Rhizobium* deep in the soil, providing protection from climatic fluctuations.

The current study compares a granular inoculant formulation for alfalfa to traditional inoculation methods including pre-inoculation of seed and on-site inoculation with a peat-based formulation and the untraditional on-site inoculation of oat seed. The project is a field study performed at three sites in the Black and Grey soil zones over a three year period. Results are presented for the establishment year and the first cut of the first production year.

Materials and Methods

Establishment year (1999):

Three field sites were established in the spring of 1999 at the Seager Wheeler (SW) farm near Rosthern, the Conservation Learning Centre (CLC) south of Prince Albert, and on producer's land near Tisdale (TIS).

The site at Tisdale was established using canola (46A73; Pioneer Hi-Bred Production Ltd.) as the cover crop and was seeded May 25, 1999. Canola is a typical companion crop for "dehy" alfalfa production. Flax was grown on the site in 1998. Individual treatment plots were 2.6-m x 11-m. The experimental design was a randomized complete block design with four replications. Treatments consisted of: 1, uninoculated alfalfa *(Medicago sativa* cv. Beaver; this variety was used for all of the inoculated treatments) – control; 2, pre-inoculated alfalfa; 3, on-site inoculated alfalfa (peat-based inoculant applied at seeding); 4, alfalfa + granular inoculant banded (ca. 1 inch below the seed row); 5, granular inoculant placed with the alfalfa seed; and 6, on-site inoculated alfalfa hand-broadcast and raked in. A disc seeder with 8-inch row spacing was used to seed the plots. Alfalfa was seeded at a rate of 7 lb acre^{-1}; canola at 5 lb acre^{-1}; and the granular inoculant applied at a rate of 7 lb acre^{-1}. Plots were seeded in two passes: canola and phosphate fertilizer (20 lb acre^{-1}, 12-54-0) were placed in the first pass; alfalfa seed and the inoculant in the second pass. Canola seed was placed approximately ¼ inch deep and phosphate 1 inch to the side and below the seed. Alfalfa was seeded approximately ¼ inch deep. Depending on the treatment, granular inoculant was placed with the alfalfa seed or banded at the depth of the fertilizer. Plots were sprayed post-emergently with Pursuit according to manufacturer's recommendations.

The Seager Wheeler site was seeded May 26th, 1999. This site was established as a typical hay production site, using oat (*Avena sativa*) as the companion crop. A triticale/pea mix was grown on the site in 1998. The plot size and seeding equipment were the same as those used at the Tisdale site. The same treatments as the Tisdale site were seeded except that oat was seeded instead of canola as the companion crop. In addition, instead of the broadcast treatment of on-site inoculated alfalfa (treatment 6) the oat seed was inoculated on-site using a peat-based inoculant. The oat seed was placed approximately 1 inch deep and was seeded at a rate of 40 lb acre^{-1}. Plots were seeded in two passes. In the first pass the oat and phosphate fertilizer (20 lb acre^{-1}, 12-54-0) were placed. Alfalfa seed and the inoculant were placed in the second pass. Seed and fertilizer placement depths as well as plot sizes and seeding equipment were the same as for the Tisdale site. Plots were sprayed post-emergently with Pardner according to the manufacturer's recommendations.

The CLC site was seeded June 2nd, 1999. Like the Seager Wheeler site, this site was established as a typical hay production site, using oat as the cover crop. Barley was grown on the site in 1998. This site was a minimum till site. Several weeks before seeding the field was sprayed with Roundup transorb. Alfalfa and oat were seeded directly into the barley stubble. Plots were seeded using an Edwards hoe drill with 8 inch row spacing. The size of individual treatment plots was 4-m x 10-m. The treatments including seeding rates, and seed and fertilizer placements were the same as those used at the Seager Wheeler site. Plots were seeded in two passes. The oat seed and phosphate fertilizer (20 lb acre^{-1}, 12-54-0) were placed in the first pass. Alfalfa seed and inoculant were placed in the second pass. Depending on the treatment, granular inoculant was placed with the alfalfa seed or side-banded at the depth of the fertilizer/oat. No post-emergent herbicide was deemed necessary.

All of the inoculant formulations were prepared from the same batch of *Rhizobium meloliti* by MicroBioRhizogen Corporation, Saskatoon.

Biological nitrogen fixation was quantified using the ^{15}N dilution technique. Two to three weeks after seeding ^{15}N-labelled NH_4NO_3 (3.75 lb acre^{-1} 13.3 atom %) was applied to 1-m^2 microplots within each treatment plot. Subsamples of shoot tissue were harvested from these microplots 8 weeks later. Shoot tissue was oven dried (65°C) for 48 hours, ground with a cyclone mill and subsequently reground in a rotating ball-bearing mill. Subsamples were analysed by mass spectrometry for ^{15}N content and the amount of N derived from atmosphere calculated. Bromegrass was used as the reference crop.

Nodulation was scored by digging up the root system of 3-5 plants from each plot. Excess soil was removed, the roots bagged in plastic and returned to the lab for scoring. At the lab, soil was rinsed from the root system and numbers, sizes, and pinkness of nodules scored. Details of the scoring system are outlined in the footnote of Table 1. The system for scoring nodulation was adapted from a similar nodulation scoring system for field pea obtained from Agriculture Canada.

Stand establishment was determined by counting the number of emerged alfalfa plants in a 1 m^2 area. The m^2 frame was positioned to span three crop rows. Nodulation scoring, stand establishment and ^{15}N subsampling was done the third week of July at all of the sites. Biomass was measured by harvesting plants in a 1 m^2 area. Plants were harvested the last week of August, dried (65°C) and weighed.

First production year (2000):

Stand counts, nodulation scoring, ^{15}N subsampling and m^2 biomass sampling was done at all sites the third week of June, 2000. This sampling took place within one week of harvesting the first cut. All of the procedures followed were the same as for the 1999 sampling.

Results and Discussion

Inoculation treatment affected nodulation scores at the Conservation Learning Centre and Seager Wheeler sites in 1999, but differences had disappeared by the first harvest in 2000 (Table 1). At the Conservation Learning Centre 1999 site, the two granular inoculants along with the site inoculated oat seed all had the highest nodulation scores. Those treatments where the inoculant was placed on the seed coat had the lowest scores. In contrast, at the Seager Wheeler 1999 site, the granular inoculant placed in the alfalfa seed row had the lowest nodulation scores. At this site, the seed applied inoculants all resulted in the highest nodulation scores. At Tisdale there were no differences in nodulation scores as a result of inoculation treatment. One shortcoming of the nodulation scoring system is that it does not incorporate any measure of where on the root system the nodules occur. Furthermore, in this study nodulation scores do not accurately predict biological nitrogen fixation (Table 2). Plants at the CLC and Seager Wheeler sites showed no difference in biological nitrogen fixation even though they did show differences in nodulation. In contrast, at Tisdale, no difference in nodulation scores was observed associated with the inoculation treatments, however, biological nitrogen fixation did show a treatment response. At this site the control plants had lower percent nitrogen derived from atmosphere than plants in all of the treatments except the granular banded and broadcast alfalfa treatments.

Table 1. Nodulation scores[1] (n=12) for alfalfa inoculated with different delivery formulations of *R. meliloti*.

Treatment	------ CLC ------		------- SW -------		------- TIS -------	
	1999	2000	1999	2000	1999	2000
Control	0.0	2.8	4.0	2.6	2.5	3.0
Pre-inoc. alfalfa	2.0	2.8	5.0	3.3	3.0	3.0
Site inoc. alfalfa	1.0	3.6	4.0	3.6	3.0	3.4
Granular banded	3.0	3.0	3.0	2.4	3.0	3.2
Granular seed placed	2.5	3.1	1.5	3.5	2.0	3.0
Site inoc. oat	2.5	2.3	3.0	2.4	na[2]	na
Broadcast alfalfa	na	na	na	na	4.0	3.0
ANOVA			*Probability*			
Replicate	0.662	0.229	0.715	0.012	0.157	0.005
Treatment	0.003	0.656	0.002	0.108	0.141	0.729

[1] Nodulation was scored using a 0-5 scale that assessed numbers of pink nodules on the root systems of 5 plants. A score of 5 was given to a sample with >30 pink nodules, a score of 0 was given if no pink nodules were found.

[2] Indicates that the treatment was not at the site.

Table 2. Biological N_2 fixation for alfalfa inoculated with different delivery formulations of *R. meliloti*. Only data from the establishment year is presented.

Treatment	------ CLC ------	------- SW -------	------- TIS -------
	1999	1999	1999
Control	53.5	37.2	36.6
Pre-inoc. alfalfa	48.6	33.7	44.2
Site inoc.alfalfa	50.4	39.5	44.1
Granular banded	53.3	38.3	41.1
Granular seed placed	50.3	38.7	45.1
Site inoc. oat	53.4	36.0	na[1]
Broadcast alfalfa	na	na	41.2
ANOVA		*Probability*	
Replicate	0.004	0.001	0.001
Treatment	0.560	0.220	0.036

[1] Indicates that the treatment did not appear at the site

Stand establishment at the Conservation Learning Centre site was affected by the inoculation treatment (Table 3). All of the plots where the inoculant was placed with the alfalfa seed had very poor stand counts. In those treatments where the inoculant was placed deep in the soil stand establishment was good. Exactly how inoculant placement can affect stand establishment is unknown. The Conservation Learning Centre was the only minimum till site of the three, and was seeded using a different seeder: an Edwards hoe drill rather than the disc seeder used at Seager Wheeler and Tisdale. Rather than a direct inoculant effect, the treatment effects may have been due to an incompatibility of the seeder with the inoculated seed. For example, flow of inoculated alfalfa seed through the seeder may have been inhibited. However, this would not explain the poor performance of the granular seed placed inoculant. By the 2000 season, any statistically significant differences in stand counts as a result of inoculant treatment had disappeared. However, the on-site inoculated alfalfa and granular seed placed inoculant treatments still tended to be the lowest in terms of stand establishment. Neither of the other two sites showed differences in stand establishment associated with inoculation treatment.

The poor stand establishment associated with seed placed inoculant treatments at the Conservation Learning Centre 1999 site, translated into poor forage yields (Table 4). Treatments where the inoculant was placed below the alfalfa seed row had the highest yields. Despite the very poor performance of some of the treatments in 1999, differences in yield had disappeared by the first harvest of the 2000 growing season. The Seager Wheeler and Tisdale sites also showed treatment related differences in forage yield in 1999. At both of these sites, plots where the granular inoculant was banded into the soil were among the highest yielding. However, at Seager Wheeler, the benefit of this treatment was not simply the deep placement, because the plots where the inoculant was placed deep in the soil via inoculation of the oat seed were among the poorest producing. At the Tisdale 1999 site the only treatment that resulted in relatively poor yields was the broadcast alfalfa treatment. Except for the site inoculated alfalfa treatment, all of the other treatments resulted in yields that were higher than the control plots. Once again, by the 2000 growing season yield differences associated with inoculant treatments were no longer apparent.

Table 3. Mean alfalfa stand establishment (no. stems/m^2) for alfalfa inoculated with different delivery formulations of *R. meliloti*.

Treatment	------ CLC ------		------- SW -------		------- TIS -------	
	1999	2000	1999	2000	1999	2000
Control	21	68	28	110	22	104
Pre-inoc. alfalfa	2	62	27	100	31	111
Site inoc.alfalfa	3	28	23	96	23	105
Granular banded	25	62	34	81	16	83
Granular seed placed	2	44	42	109	23	123
Site inoc. oat	36	67	28	107	na[1]	na
Broadcast alfalfa	na	na	na	na	17	98
ANOVA			*Probability*			
Replicate	0.174	0.485	0.632	0.694	0.461	0.630
Treatment	0.000	0.154	0.362	0.382	0.332	0.116

[1] Indicates that the treatment did not appear at the site

Table 4. Forage yield (g m^{-2}) for alfalfa inoculated with different delivery formulations of *R. meliloti*.

Treatment	------ CLC ------		------- SW -------		------- TIS -------	
	1999	2000	1999	2000	1999	2000
Control	28	295	33	307	35	185
Pre-inoc. alfalfa	3	291	31	280	58	174
Site inoc. alfalfa	7	158	45	259	37	161
Granular banded	46	316	48	280	55	188
Granular seed placed	4	274	38	273	44	152
Site inoc. oat	58	290	28	229	na[1]	na
Broadcast alfalfa	na	na	na	na	17	138
ANOVA			*Probability*			
Replicate	0.174	0.040	0.224	0.593	0.024	0.078
Treatment	0.000	0.124	0.016	0.445	0.046	0.220

[1] Indicates that the treatment did not appear at the site

Conclusions

In contrast to theory, granular inoculants were not consistently superior to pre-inoculated and self-sticking peat inoculant formulations. Treatment differences were detected only in the establishment year for any of the productivity parameters and had disappeared by the mid-season cut of the first production year. Analysis of biological nitrogen fixation may still show a effect of inoculation treatment.

Acknowledgements

Funding was provided by the Saskatchewan Agricultural Development Fund. Inoculants were provided by MicroBioRhizogen Corporation of Saskatoon and canola seed by Pioneer Hi-Bred

Production Ltd., Research and Product Development, Georgetown, Ontario. Technical assistance was provided by B. Miller, M. Korol and A. Theede.

Literature Cited

Bezdicek, D.F., D.W. Evans, B. Abeda and R.E. Witters. 1978. Evaluation of peat and granular inoculum for soybean yield and N fixation under irrigation. Agron. J. 70:865-868.

Clayton, G., W. Rice, S. Blade, C. Grant, N. Harker, A. Johnston, G. Lafond, & M. Collins. 1996. Proc. Pulse Res. Workshop. Nov. 28-29, Calgary, AB, pp. 16-17.

Muldoon, J.F., D.J. Hume and W.D. Beversdorf. 1980. Effect of seed and soil-applied Rhizobium japonicum inoculants on soybeans in Ontario. Can. J. Plant Sci. 60: 399-409.

Saskatchewan Agriculture and Food. 1998. Forage establishment: getting it to grow. <http://www.agr/gov.sk.ca/saf/crops/foragee1.htm >Accessed Oct. 15, 1998.

Smith, R.S. 1992. Legume inoculant formulation and application. Can. J. Microbiol. 38:485-492.